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RESEARCH ARTICLE

Methicillin resistant *Staphylococcus aureus* nasal colonization among secondary school students at Duhok City-Iraq

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ABSTRACT

Objective: Methicillin-resistant *Staphylococcus aureus* (MRSA) widely distributed in hospitals around the world. There is strong relationship between disease development and *S. aureus* nasal carriage. The aim of this study was to evaluate the prevalence and epidemiology of nasal colonization with *S. aureus* and MRSA in the community of Duhok city, Iraq.

Methods: 489 students aged 16 to18 years were included. Nasal swab samples were collected followed by antimicrobial susceptibility test. MRSA isolates were selected and investigated for the *mecA* gene. Also the prevalence of Panton-Valentine Leukocidin (PVL) gene was also studied.

Results: A total of 90 (18.4%) out of 489 (18.4%) of the students were found to be colonized by *S. aureus*. Only 10 (2.04%) of the students were found to be MRSA carrier. All MRSA isolates were sensitive to Vancomycin. PLV gene was detected in one MRSA strain.

Conclusion: This is the first study investigating *S. aureus* colonization in students in the Duhok city. Nasal carriage of *S. aureus* and MRSA is comparable with reports from elsewhere. Fortunately, all trains included in our study were sensitive to vancomycin. Further research is needed to examine the *SCCmec* elements and the evolution of MRSA over the time. *J Microbiol Infect Dis 2014;4(2): 59-63*

Key words: Methicillin-resistant *Staphylococcus aureus*, *Staphylococcus aureus*, community acquired-MRSA, Vancomycin, Iraq

Irak'ın Duhok şehrinde ortaokul öğrencilerinde metisiline dirençli Staphylococcus aureus ile burun kolonizasyonu

ÖZET

Amaç: Metisiline dirençli *Staphylococcus aureus* (MRSA) tüm dünyada hastanelerde ve yoğun bakım ünitelerinde yaygın şekilde bulunmaktadır. Burunda taşıyıcılık ile MRSA'ya bağlı hastalık gelişmesi arasında belirgin bir ilişki bulunmuştur. Bu çalışmanın amacı Irak'ın Duhok şehrinde toplumda *S. aureus* ve MRSA burun kolonizasyonunun prevalansını ve epidemiyolojik özelliklerini belirlemektir.

Yöntemler: Çalışmaya yaşları 16-18 arasında değişen 489 öğrenci dahil edildi. Burun sürüntü kültürleri alındı ve antimikrobiyal duyarlılık testleri yapıldı. MRSA izolatları seçilerek bunlarda *mecA* geninin varlığı araştırıldı. Yanısıra bu izolatlarda Panton-Valentin Lökosidin (PVL) geninin varlığı da araştırıldı.

Bulgular: Çalışmayan alınan 489 öğrenciden 90'ında (% 18,4) *S. aureus* ile kolonizasyon saptandı. Bunlardan sadece 10'unda (% 2,04) MRSA taşıyıcılığı belirlendi. Tüm MRSA izolatları vankomisine duyarlı idi. PLV geni sadece bir MRSA izolatında belirlendi.

Sonuçlar: Bu çalışma Duhok şehrinde öğrencilerde *S. aureus* kolonizasyonunu araştıran ilk çalışmadır. Burunda *S. aureus* taşıyıcılığı ve MRSA oranları farklı merkezlerle benzer oranda bulunmuştur. *SCCmec* öğelerini araştırmak ve zaman içerisinde MRSA yayılımını araştırmak için ileri çalışmalara ihtiyaç olduğu görülmektedir.

Anahtar kelimeler: Metisiline dirençli *Staphylococcus aureus*, MRSA, *Staphylococcus aureus*, toplumda kazanılmış MRSA, Vancomisin, Irak

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INTRODUCTION

Staphylococcus aureus is considered to be one of the most important resistant pathogen and it was one of the earliest microorganisms in which penicillin resistance was detected.[1] Pencillin-resistant *S. aureus* became a major threat in hospitals in the 1950s leading to the use of methicillin.[2] However, methicillin resistant strains appeared amongst *S. aureus* strains isolated from hospitals in 1961.[3] Methicillin-resistant *S. aureus* (MRSA) has become one of the most important hospitals and community acquired causative agents of human skin infections and invasive diseases, such as pneumonia, endocarditis, deep abscess formation, osteomyelitis, and septic arthritis.[2,4] Colonisation or infection of MRSA was generally considered to be hospital associated (HA-MRSA).[4] However, strains of MRSA have emerged in the community and often known as community-associated MRSA (CA-MRSA).[5] CA-MRSA differs from HA-MRSA as CA-MRSA is likely to be more susceptible to other antibiotics such as trimethoprim–sulfamethoxazole (TMP/SMX).[6] Additionally, CA-MRSA carries staphylococcal cassette chromosome methicillin resistant locus (*SCCmec*) type IV or V.[7] It is documented that a major predisposing factor for succeeding infection and transmission of MRSA is the presence of this pathogen in the nose.[1,8] Some publications suggested that the percentage of nasal carriers MRSA adults is about 7%.[9] Further studies showed that 10% to 35% of populations harbour MRSA in the nose transiently or persistently and up to 30% of healthy people have *S. aureus* in their nose or other body areas.[10] In a study conducted in the United States, the nasal carriage rate of CA-MRSA in children increased from 0.6% in 2001 to 1.3% in 2004.[11] Several studies have been conducted with medical students,[12,13] but few studies have examined the characteristics of CA-MRSA in a general student population.[14,15] The purposes of this report were to evaluate the prevalence of nasal carriage of *S. aureus* and MRSA, to study the prevalence of PVL and to examine the vancomycin sensitivity pattern amongst student population in Duhok city, Iraq.

METHODS

Sample collection

To ensure adequate sample size, 489 secondary school students in Duhok city were included in the study, aged 16 through 18 years. In order to fulfil the case definition of CA-MRSA infection according to the Center for Disease Control and Prevention (CDC), those students with one of the following HA-MRSA risk factors were excluded: (1) diagnosis of MRSA infection within two days of hospitalization, (2) Previous admission to hospital, undergoing surgical procedures, renal dialysis and long-term stay in a care unit within a year of the nasal swab taking, (3) Using of an catheter or intravenous line at the time of swab taking (4) Former detection of MRSA from the subject. 16 Also the included students should not have received antibiotics recently, should not have lived with a person who has chronic disease that need frequent hospital visits and should not have received care for wound infection. The study was conducted with the approval of ethics committee in the University of Duhok, School of Medicine.

Cultural and biochemical identification of MRSA and antimicrobial susceptibility testing

Nasal swabs were taken from both anterior nares of the students. To avoid or minimize the mild irritation that may happen by dry swabs, swabs were moistened with sterile distilled water.[10] The swab was inserted about 2 cm, with rotating 3 seconds, into the naris. The same swab was used for other naris. Then, the swabs were directly cultured in Mannitol Salt Agar (Oxoid), and incubated at 35ºC for 48 hours. The strains were considered *S. aureus* relying on mannitol salt agar fermentation, Gram stain, morphology, catalase test and coagulase test. Antimicrobial susceptibility testing to antimicrobial agents including oxacillin and vancomycin was performed by the Kirby-Bauer disk diffusion and agar dilution assay methods, vancomycin sensitivity was tested on Muller-Hinton agar (Oxoid Limited, Hampshire, England), according to the Clinical Laboratory Standards Institute (CLSI) recommendations. Further investigations were performed for MRSA isolates, including polymerase chain reaction (PCR) assays for the *mecA* gene and the genes that encode Panton-Valentine leukocidin (PVL).

Extraction of chromosomal DNA and Polymerase chain reaction (PCR)

Qiagen DNA Purification kit was used to extract DNA from *S. aureus* isolates according to the manufacturer's instructions (Qiagen). Existence of the *mecA* gene was investigated in all MRSA isolates were examined. PCR was used to confirm the presence of the *mecA* gene as described by Murakami et al.17 Two previously described primers MR1 (5'GTGGAATTGGCCAATACAGG3') and MR2 (5'TAGGTTCTGCAGTACCGGAT3'),[18] were utilised

to amplify *mecA*. Amplification conditions for *mecA* were: denaturation at 95 0C for 30 s, annealing at 58 0C for 1 min, extension at 72 0C, denaturation for 1 min at 95 0C, (for 30 cycles), and a final extension for 5 min at 72°C. Luk-PV-1(5'ATCATTAGG TAAAATGTCTGGACATGATCCA3') and Luk-PV-2 (5'GCATCAAGTGTATTGGATAGCAAAAGC3') primers19 were utilized to amplified the PVL gene using standard protocols as following: 1 min for initial template denaturation step at 95 °C, annealing at 55°C for 1 min, primer extension at 72 °C for 2 min, and denaturation at 94 °C for 45 s, for 35 cycles, with 5 min as final extension at 72 °C. All PCR reactions were performed, using a C1000 model Thermal Cycler (BIO-RAD), in a 25 µl final volume containing 1 µl of *S. aureus* DNA, 1 µl primer (5 µM), 0.5 µl (1.5 U) of Taq DNA polymerase (Roche), 200 µM of each dNTP (Deoxyribonucleotide mix, 10 mM each), 0.5 µl dNTP, and 2.5 µl 10x PCR buffer. 1.5% agarose gel electrophoresis (80 V in 1× TAE buffer) was used to analyse the PCR products. The gel was stained with 10 µg ml-1 ethidium bromide (Sigma) and visualized using an ultraviolet trans-illuminator to detect the DNA. DNA ladder 100bp (Gibco, Paisley, UK) was run alongside the samples to enable analysis of DNA fragment size in the samples.

RESULTS

S. aureus was detected in 90 (18.4%) of 489 students carried *S. aureus*. Only 10 (2.04%) students were found to be MRSA carrier. All MRSA isolates were sensitive to vancomycin.

Genetic resistance to methicillin was verified by PCR for detection of the *mecA* gene. PCR data obtained showed that all isolates of MRSA typed positive for *mecA* gene. Only one isolate of MRSA carried the PLV gene.

DISCUSSION

S. aureus is a common inhabitant of the upper respiratory tract and responsible for common infections.[20] *S. aureus* from the nose was shown to be associated with community and hospital associated infections.[4] In healthy population, the nasal carriage rate *S. aureus* is 10% to 35% and this rate can be influenced by age, underlying illness, and the environment in which the person lives.[21] In our study, student nasal carriage rate of *S. aureus* was shown to be 18.4%. This result is comparable to previous studies from different countries such as Taiwan.[22] However, the carriage rate in our study was shown to be lower than what has been found in other community-based nasal carriage studies.[23] For example in northern Pakistan, the nasal colonisation of *S. aureus* was documented in 86 out of 360 students (24%).[7] In the United States from 2003-2004, the *S. aureus* carriage rate in the civilian non-institutionalized population according to the National Health and Nutrition Examination Survey was 28.6%.[24] In India, the nasal carriage rate of *S. aureus* was 13% 25 while it was only 12.6% in Sudan.[10]

In this study, we found that the nasal carriage of MRSA among the *S. aureus* isolates was 11.1% and the overall rate of MRSA among the students was 2.04%. In agreement with our results, *S. aureus* was isolated from nasal swabs of 102 (40.8%) of the 250 volunteers, 2.4% of them were CA-MRSA carriers in Brazil.[26] In Palestine in 2011, the nasal carriage of MRSA among the students was 2.5%.[7] In 2010, a total of 322 University students in Taiwan were screened and 2.2%, of them harboured MRSA.[22] In Pakistan, from 2007 to 2008, MRSA from nasal swabs from anterior nares was 1.5%.[27] In 2001-2002, national MRSA colonization prevalence in USA was 0.8%.[8] On the other hand, the nasal colonisation of MRSA was lower than what was found in other studies. For example, in a study conducted in India in 2009, the anterior nares colonisation of MRSA was 15.4%.[25] In addition, MRSA in different studies for different regions of Iran varied from 20.48% to 90%.[28] A total of 40 (33.3%) *S. aureus* strains were isolated from the nasal swabs screened in Nigeria, the overall MRSA was 27.5%.[4]

Vancomycin is considered one of the last options of antibiotic for cure of *S. aureus* infections that are resistant to other antibiotics. However, with the antibiotic pressure applied by the mounting utilisation of vancomycin in the management of MRSA infections, resistant strains to vancomycin, known as vancomycin-resistant *S. aureus* (VRSA), have been reported. Fortunately, no vancomycin-resistant strain was found among *S. aureus* isolates in this report. Interestingly, the sensitivity to vancomycin has been found in virtually all isolates of *S. aureus* in many studies worldwide.[7,10] Thus vancomycin could be a wise choice for the management of CA-MRSA.

PVL is the most well-known virulence factor of CA-MRSA. The severest S. aureus-related symptoms and diseases are attributed to the presence of the PVL toxin such as furunculosis, severe necrotising pneumonia, necrotic lesions of the skin and soft tissues and it is also can destroy the cell membrane of white blood cells.[29] PVL is thought to be associ-

ated partially to high virulent MRSA strains in the community.[30] There is now considerable molecular evidence indicates that the CA-MRSA strains have developed in the community by the horizontal acquirement of *SCCmec* elements and PVL genes.[31] The PVL positivity among our CA-MRSA was 10% (only in 1 out of 10 MRSA isolates). Although PVL can consistently found among CA-MRSA strains [32] it has been reported that it is less often found in CA-MRSA strains linked to asymptomatic nasal colonization.[33] Previous studies have revealed that approximately 8-75% of the MRSA nasal isolates carry the PVL gene.[7,8] Additionally, the percentage of PVL gene was 70-100% among skin isolated MRSA.[32]

To conclude, nasal carriage of *S. aureus* and MRSA is comparable with reports from elsewhere. Actions should be taken to keep the emergence and transmission of these strains to a minimum. Fortunately, all isolates were sensitive to vancomycin. Further research is needed to examine the *SCCmec* elements and the evolution of MRSA over the time.

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